

Appl. No. 09/643,755
Amdt. Dated May 27, 2004
Reply to Office action of December 16, 2003

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for the production of chymosin in a plant seed comprising:

a) introducing into a plant cell a chimeric nucleic acid sequence molecule comprising in the 5' to 3' direction of transcription:

- 1) a seed-specific promoter capable of regulating transcription in said plant cell operatively linked to;
- 2) a second nucleic acid sequence encoding a chymosin polypeptide operatively linked to;
- 3) a third nucleic acid sequence capable of terminating transcription in said plant cell;

b) growing said plant cell into a mature plant capable of setting seed wherein said seed contains chymosin; and

c) obtaining seed from the mature plant wherein the seed contains at least 0.5% (w/w) chymosin; and

(d) isolating said chymosin from said seed using a method comprising:

- (i) crushing the plant seed to obtain crushed plant seed;
- (ii) fractionating the crushed plant seed into an oil fraction, aqueous fraction and a fraction comprising insoluble material;
- (iii) contacting the aqueous fraction with a protein binding resin; and
- (iv) recovering chymosin from the protein binding resin.

Claim 2 (deleted).

Appl. No. 09/643,755

Amdt. Dated May 27, 2004

Reply to Office action of December 16, 2003

Claim 3 (previously amended): The method according to claim 1 wherein said seed-specific promoter is a phaseolin promoter.

Claim 4 (deleted).

Claim 5 (original): The method according to claim 1 wherein the second nucleic acid sequence encoding a chymosin polypeptide comprises a nucleic acid sequence encoding a chymosin pro-peptide, a nucleic acid sequence encoding a chymosin pre-peptide or a nucleic acid sequence encoding chymosin pre-pro-peptide.

Claim 6 (original): The method according to claim 5 wherein the second nucleic acid sequence encoding a chymosin polypeptide further comprises a nucleic acid sequence encoding a plant signal sequence.

Claim 7 (original): The method according to claim 1 wherein the second nucleic acid sequence encoding a chymosin polypeptide further comprises a nucleic acid sequence encoding a plant signal sequence.

Claim 8 (previously amended): The method according to claim 7 wherein the plant signal sequence is a tobacco PR-S signal sequence.

Claim 9 (original): The method according to claim 8 wherein the nucleic acid sequence encoding chymosin linked to a PR-S signal sequence comprises a nucleic acid sequence as in SEQ.ID.NO.:1.

Claim 10 (original): The method according to claim 1 wherein said third nucleic acid sequence is a phaseolin terminator.

Claim 11 (previously amended): The method according to claim 1 wherein the chymosin is a mammalian chymosin obtained from a bovine, sheep or goat source.

Appl. No. 09/643,755

Amdt. Dated May 27, 2004

Reply to Office action of December 16, 2003

Claim 12 (original): The method according to claim 6 wherein codon usage for said nucleic acid sequence encoding chymosin, chymosin pro-peptide, chymosin pre-peptide and chymosin pre-pro-peptide has been optimized for use in plants.

Claim 13 (original): The method according to claim 1 wherein said plant is selected from the group of plants consisting of soybean (*Glycine max*), rapeseed (*Brassica napus*, *Brassica campestris*), sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), corn (*Zea mays*), tobacco (*Nicotiana tobacum*), alfalfa (*Medicago sativa*), wheat (*Triticum sp.*), barley (*Hordeum vulgare*), oats (*Avena sativa L.*), sorghum (*Sorghum bicolor*), *Arabidopsis thaliana*, potato (*Solanum sp.*), flax/linseed (*Linum usitatissimum*), safflower (*Carthamus tinctorius*), oil palm (*Elaeis guineensis*), groundnut (*Arachis hypogaea*), Brazil nut (*Bertholletia excelsa*) coconut (*Cocos nucifera*), castor (*Ricinus communis*), coriander (*Coriandrum sativum*), squash (*Cucurbita maxima*), jojoba (*Simmondsia chinensis*) and rice (*Oryza sativa*).

Claim 14 (original): The method according to claim 1 wherein at least 1% (w/w) of said total seed protein is chymosin.

Claim 15 (original): The method according to claim 1 wherein at least 2% (w/w) of said total seed protein is chymosin.

Claim 16 (original): The method according to claim 1 wherein at least 4% (w/w) of said total seed protein is chymosin.

Claim 17 (currently amended): A method for the production of plant seeds containing at least 0.5% (w/w) chymosin in the total seed protein comprising:

(a) introducing into each of at least two plant cells a chimeric nucleic acid sequence molecule comprising in the 5' to 3' direction of transcription:

Appl. No. 09/643,755

Amtd. Dated May 27, 2004

Reply to Office action of December 16, 2003

- 1) a seed-specific promoter capable of regulating transcription in said plant cell operatively linked to;
- 2) a second nucleic acid sequence encoding a chymosin polypeptide operatively linked to;
- 3) a third nucleic acid sequence capable of terminating transcription in said plant cell;

- (b) growing each plant cell into a mature plant capable of setting seed;
- (c) obtaining seed from each mature plant;
- (d) detecting the levels of chymosin in the seed of each plant obtained in step (c) or in the seed of a plant generated from the seed of a plant obtained in step (c); and
- (e) selecting plants that contain at least 0.5% (w/w) chymosin in the total seed protein; and

(f) isolating said chymosin from said seed using a method comprising:

- (i) crushing the plant seed to obtain crushed plant seed;
- (ii) fractionating the crushed plant seed into an oil fraction, aqueous fraction and a fraction comprising insoluble material;
- (iii) contacting the aqueous fraction with a protein binding resin; and
- (iv) recovering chymosin from the protein binding resin.

Claims 18-20 (deleted).

Claim 21 (currently amended): A method according to claim 19 1 wherein said protein binding resin is a hydrophobic interaction resin.

Claim 22 (currently amended): A method according to claim 20 17 wherein said protein binding resin is a hydrophobic interaction resin.

Claim 23 (original): A method according to claim 22 further comprising using an ion exchange resin to further purify the chymosin.

Appl. No. 09/643,755
Amdt. Dated May 27, 2004
Reply to Office action of December 16, 2003

Claims 24-28 (deleted).